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Spatial and temporal differences in gonad development, sex ratios and reproductive output, influence the sustainability of exploited populations of the European oyster, *Ostrea edulis*

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Abstract

1. The European native oyster, *Ostrea edulis*, has been in severe decline since the early 1900s across Europe with many fisheries now declared commercially extinct. In light of this broad scale population decline, the UK has listed *O. edulis* as a threatened species, requiring conservation action under the Biodiversity Action Plan (BAP). In addition to this designation, in Scotland *O. edulis* beds are search features (SF) and priority marine features (PMF) for MPA site selection. These sites are also listed as a feature of conservation importance and included on the OSPAR list of threatened/declining species and habitats.
2. Recent studies have identified *O. edulis* populations with heavily male-skewed sex ratios, which may have contributed to fisheries decline due to reduced levels of fertilisation. This species is a protandrous alternating hermaphrodite and individuals may change sex in response to local conditions. This study aimed to assess how sex ratios vary temporally

and if this is correlated with temperature, by studying two exploited populations in Loch Ryan, Scotland and Chichester Harbour, England.

3. This study suggests that the proportion of male phase oysters is positively correlated with water temperature and that the study population in cooler waters had a more balanced sex ratio overall (the Loch Ryan population was significantly similar to 1:1 for 10 of 13 months, whereas in Chichester only 1 of the 7 months was significantly similar).
4. This study provides evidence to suggest that a critical temperature threshold for sex determination exists in *O. edulis* and for the Loch Ryan population we suggest that this is 16.5°C. However, further work is required to assess how this threshold may change between sites and how future climate change scenarios might affect the sex ratio of native oyster populations.

Keywords

Aquaculture, Climate change, Coastal, Invertebrates, Reproduction, Restoration, Sea loch

1. Introduction

Native oyster populations, which historically dominated many coastal and estuarine ecosystems, have been driven globally to the brink of functional extinction by overexploitation (Beck et al., 2011). Populations of oysters, such as the European oyster *Ostrea edulis* (Linnaeus), continue to be harvested unsustainably due to adherence to traditional methods of exploitation (Beck et al., 2011) and a failure to apply understanding of the biology of the species to its management. Due to the decline of *O. edulis* it is now listed as a UK Biodiversity Action Plan (BAP) species, to be maintained and expanded (UKBG, 1999), and is included on the OSPAR list of species and habitats that were threatened and/or declining in 2003 (OSPAR, 2009). Within Scotland, *O. edulis* beds are priority marine features (PMF) and search features (SF) for MPA site selection. Since this species

remains important to fisheries around the UK, conservation aims need to sustain local populations whilst maintaining economically viable fisheries (Lane & Stephenson, 1995). Loch Ryan contains two sites of Special Scientific Interest (SSIs) due to geological interest (Mather, 2003) and in 1990 the whole loch was designated as a Marine Consultation Area (MCA) by Scottish Natural Heritage (SNH), highlighting it as an area distinct for the sensitivity and quality of its marine environment (Golding, Alman & Service, 2001; Loch Ryan Advisory Management Forum, 1999). Chichester Harbour is situated within an Area of Outstanding Natural Beauty (AONB) and is a site of Special Scientific Interest (SSI) partly due to the vital areas for overwintering birds.

Ostrea edulis is a protandrous alternating hermaphrodite (Orton, 1937) which first reproduce as a male on reaching sexual maturity and thereafter alternates between sexes. The male gametes are released into the water column and drawn into the female mantle cavity via the inhalant siphon, where the eggs are fertilised (Walne, 1974) and brooded for 6-8 days (Helm & Bourne, 2004). The process of sex change and the controls for sex alternation are not precisely known for *O. edulis* (Kamphausen, Jensen, & Hawkins, 2011); however, like most bivalve species it is understood to be largely controlled by neuropeptides and steroids (Morishita, Furukawa, Matsushima, & Minakata, 2010). This change in sex, or serial development of gonads in alternating sexes, occurs repeatedly throughout the lifetime of an individual and may take place several times within a single summer breeding season (Walne, 1974). Thus, the sex ratio in a population could fluctuate significantly during the breeding period with individuals reproducing as both male and female in a single season. Despite this fluctuation within a population it is suggested that the overall sex ratio should conform to a 1:1 ratio (Kamphausen et al., 2011); however, male biased ratios are frequently reported (e. g. Bourne, 1890; Cole, 1939; Kamphausen et al., 2011). Skewed sex ratios can lead to inbreeding, limited fecundity, and, coupled with exploitation, rapidly result in severe population declines.

Temperature is known to have a major effect on reproduction in *O. edulis* directly by determining the start and end of the breeding period and gametogenic progression, and indirectly by regulating sex changes (Joyce, Holthuis, Charrier, & Lindegarth, 2013). Non-native oyster species in Europe have had increasing reproductive success since the 1960s partly due to increasing sea surface temperatures (Thomas et al., 2015) and the response of native species to the same environmental drivers is therefore important to predict potential competitive interactions. Most previous research to assess the impacts of temperature on sex ratios and gametogenesis in oysters has been based in laboratory or hatchery settings (Joyce et al., 2013; Mann, 1979). In a long-term laboratory study of reproduction in *O. edulis*, a female-dominated group in cooler temperatures shifted to become a male-dominated group after exposure to gradually increasing temperature conditions, indicating that there is perhaps a direct correlation to temperature rather than a simple seasonal phenology in sex changes (Joyce et al., 2013). In the present study, we aim to assess how temperature affects sex ratio and fecundity in populations of *O. edulis* under natural conditions and fisheries pressures in order to aid conservation efforts and develop sustainable fisheries. We predicted that wild populations of *O. edulis* would show similar temperature-based changes in sex ratios, with cooler spring temperatures early in the breeding season correlated with a female bias and more female oysters brooding, and warmer water temperatures in late summer and autumn leading to a male bias with fewer female oysters brooding.

To explore this potential temperature driven change in sex ratios, this study aimed to explore two relevant case studies of exploited *O. edulis* populations that span a wide latitudinal range in the UK, and which are exposed to differing temperature regimes. The two populations are located in Loch Ryan, Scotland, which was studied in the 1960s and 2012-2015 (and from now on will be referred to as LR), and Chichester Harbour, England which was studied for one year in 2011 (and from now on will be referred to as CH). We acknowledge that there are some limitations associated with comparing the one year study of CH with the more extensive studies of LR,

particularly since these investigations were not run concurrently. However, comparison of the reproductive potential of these populations provides a context to consider how temperature might affect reproductive output of oyster fisheries providing information for the sustainable management and conservation of this species.

2. Materials and Methods

2.1. Study sites

Loch Ryan, Southwest Scotland (54. 9506° N) (Figure 1) makes an interesting case study because it has fewer stressors in comparison to the fisheries on the south coast of England. Loch Ryan has no diseases (such as *bonamiasis*) present, limited boat traffic, a low fishing effort and reduced anthropogenic waste input. Loch Ryan is one of the last remaining wild European oyster fisheries in the United Kingdom that has survived long term ‘boom and bust’ cycles due to its unique private ownership over the last 300 years (Eagling, Ashton, & Eagle, 2015). The fishery in Loch Ryan was studied extensively during a closed period (1957-1970) by Millar (1963, 1964) which are examined and discussed in this study. In comparison, Chichester Harbour (50. 8056° N) in Southeast England, is known to contain diseases such as *bonamiasis*, and the area is subject to heavy commercial shipping and recreational boat traffic. In the year prior to the oyster sampling in Chichester Harbour, the oysters were concentrated from across the harbour into broodstock beds to increase the density from approximately one oyster per m² to 40 oysters per m² (Eagling, 2012; Eagling & Jensen, 2012).

2.2. Sampling

In LR, oysters were sampled (n=20) every two weeks from April through to mid-October in both 2013 (260 oysters collected in total) and 2014 (240 oysters collected in total), to compare inter and intra-annual differences within the breeding season. The oysters were collected using a 1.8 m

dredge towed at speeds of less than 2 knots by the 37 ft fishing vessel '*Vital Spark*'. In CH, sampling occurred every 2-3 weeks from April to October in 2011, using a small Van Veen Grab which enabled a total collection of 212 oysters. In the laboratory, all samples (across both sites) were first weighed wet (shell and flesh), the shell height measured with calipers, and then either dissected immediately or the shells were broken and oysters fixed and stored in 4% borax buffered formalin. When dissected, the gonad was removed and cut into two or three pieces approximately 4 mm thick. The tissue was dehydrated through a series of increasing ethanol concentrations (30, 50, 70, 90, 95 and 100%) (Meneghetti, Moschino, & Da Ros, 2004) and finally washed with Histoclear. The tissue was mounted in paraffin wax (Delgado & Pérez-Camacho, 2007) and sectioned using a Leica RM2255 microtome at 7 - 9 μ m thickness throughout the sample. Multiple slides were created for each oyster with several sections on each to ensure a clear section of the gonad. The slides were stained using an adapted Haematoxylin and Eosin procedure (Howard & Smith, 1983) to identify the gametic cells within the tissue and examined using a light microscope (Olympus BX41). The sex (predominantly in either the male or female phase, see Figure 2) and stage of gametogenic development were determined for each oyster. Gametogenic stages were scored according to the classification schemes devised by Wilson & Simons (1985) and Loosanoff (1962) where gonads were categorised into seven stages (gonad index) as in early or late development, early maturation, ripe, partially spent, spent or inactive.

The water temperature of LR was recorded every ten minutes using a Hoboware temperature logger securely tied to a creel next to the oyster beds at approximately 5 m depth and retrieved monthly to download the data. Temperature at CH was taken from a probe on the Camber Pile weather station, based about 2 nautical miles from the sampling location (accessed online from: cambermet.co.uk), just below the water surface. A temperature probe was used on four occasions to measure the temperature offset between the weather station pile and the oyster sampling location (3-5 m below the surface) and the dataset calibrated accordingly.

2.3. Data analysis

All statistical analyses were carried out in R Studio (version 0.99.903 and R version 3.3.1) (R Core Team, 2015), where the monthly sex ratio of the Loch Ryan population was compared to the suggested 'ideal' ratio of 1:1 using Chi squared tests, due to the multiple tests carried out within the hypothesis the p value was corrected using the Bonferroni correction. The annual sex ratio for 2013 and 2014 were also compared to a 1:1 ratio using Chi squared and then the 2013 ratio with the 2014 ratio. The sex ratio over both years was pooled for the Loch Ryan site and compared to other previously assessed populations in LR (1960/61) (Millar, 1963; 1964), CH (2011), Southampton, England (Kamphausen et al., 2011) and a Greek cohort (da Silva, Fuentes & Villalba, 2009).

Inter-annual temperature variation in LR was assessed using an ANOVA, and to consider the differences in brooding percentages between LR 2013 and 2014, and between LR 2013 and CH 2011, Wilcoxon tests were used. The relationship between temperature and a) sex ratio, b) brooding percentage and c) larvae per brood, and percentage males to percentage brooding females were assessed using linear regressions.

3. Results

3.1. Temperature patterns at study sites

Water temperatures in LR ranged from 1.8°C in February 1960 to 18.6°C in June 1960 (Millar, 1963), and from 4.5°C in March 2013 to 18.1°C in July 2014 during fieldwork within the present study. Temperatures within the loch were higher from July through to January in 2013/14 than 50 years previously, but were colder between February and June although there was no significant difference among the temperatures of each year (ANOVA, $f=0.64$, d.f. = 59, $p>0.05$) (Figure 3).

When comparing the results between LR and CH, the temperature was greater than 10°C from April to December 2011 in CH, and from May until November in LR. Temperatures remained

consistently above 16°C from June until mid-October only in CH and average temperatures also exceeded 18°C for 27 days in CH compared to 1 day in LR (2013/14).

3.2. Gonad development

Gametogenic changes in *O. edulis* during the breeding period (April to October) in LR showed similar patterns in both 2013 and 2014 with spawning, as evidenced by the proportion of partially and fully spent individuals, extending from June to October in both years (Figure 4). However, spawning appeared to be more advanced in 2013 when more than 40% of oysters were spawning compared to approximately 20% in June 2014 (Figure 4). In CH, individuals were identified spawning from May and a greater proportion were spent from August onwards compared to both years in LR. As the temperature and season progressed, the gonad index increased until September in LR and in CH a decrease was not observed within the sampling period (up to the end of October) (Figure 4).

3.3. Sex Ratios

The proportion of males across both sites exceeded 50% on 19 out of 23 sampling occasions in LR and exceeded 60% in all samples from CH (Figure 5). However, for the majority of months between 2013 and 2014 in LR the sex ratio was not statistically significantly different from a ratio of 1:1. For a few months each year (June 2013 and May, August, September 2014) the ratio was so heavily skewed towards a male population, that the pooled annual ratio became skewed (Table 1). Of the oysters sampled from LR in 2013, 87 displayed predominantly male characteristics and 54 female characteristics, whilst the rest were characterised as spent. This resulted in an overall 3 male: 2 female ratio among unspent animals, which differed significantly from parity (Table 1). Similarly, the sex ratio in 2014 (2 male:1 female) showed a significant departure from parity (Table 1) with 129 oysters displaying predominantly male characteristics, 58 displaying predominantly female characteristics and the remaining oysters characterised as

spent. By contrast in the 1960/61 period in LR (Millar, 1964) 9 months were significantly skewed from the 1:1 ratio (compared to the four months over 2013/14), with the greatest difference occurring in the middle of August 1961, with a ratio of 7 male: 1 female (Table 2).

Although there were four months recorded where the number of females exceeded the number of males, (September 2013, April and June 2014, and December 1960) this was not consistent between the years and so was not investigated further. When the LR 2013/14 ratios were compared between years, there was no significant difference ($\chi^2 = 1.9$, d.f. = 1, $p > 0.05$) and therefore the two years were grouped for comparison to other populations (Table 3).

3.4. Brooding

The average percent of the oyster population brooding over the breeding period (April to October) ranged from 6% (15% of females) (CH, 2011) to 18% (30% of females) (LR, 2013) (Figure 6). There was a significant difference between the percentage of brooding oysters recorded in LR 2013 and CH 2011 (Wilcoxon test $w=1$, $p < 0.05$), and in LR in 2013 and 2014 (Wilcoxon test $w=25.5$, $p < 0.05$) (Figure 6). The proportion of oysters brooding varied in CH between 3 and 10%, and in LR between 3 and 25% in 1960/61, and 5 and 25% in 2013/14 (Figure 6). Peak brooding appeared to occur around the beginning of July; a second smaller peak in brooding was identified towards the end of August in LR.

3.5. Relationships between temperature, sex ratios and reproductive output

The proportion of male oysters pooled across all sites and years showed a significant positive linear relationship with temperature (Figure 7A) ($R^2 = 0.467$ and $p < 0.05$). There was no significant relationship between the percentage of females brooding and temperature (Figure 7B) (linear regression, $p > 0.05$); however, visual inspection of these patterns suggests that the optimum temperature for brooding may be around 16.5°C for the LR population and 17.5°C for the CH population. The data recorded may also reflect two spawning periods within the

population: one at the beginning of the brooding season with a higher percentage of brooding females and then a second smaller spawning event later in the summer. When considering the abundance of larvae per oyster brood (Figure 7C), there was an apparent negative correlation with average daily sea temperature, but this was not significant ($R^2 = 0.376$, $p = 0.1$). There was no correlation between the percentage of females brooding and the percentage of male oysters (Figure 7D).

4. Discussion

4.1. Gametogenic progression

Although many studies have examined gametogenic progression in marine bivalves, including various species of oysters, these mostly focus on the influence of environmental factors or latitudinal gradients (Ruiz et al., 1992). Here we provide new insight into the inter-relationships between temperature, sex ratio, the number of brooding individuals and gametogenesis within *O. edulis*. We confirm that gametogenesis occurred throughout the summer periods, with no signs of reabsorption, suggesting that conditions in both locations monitored were suitable for oyster growth and reproduction (Kamphausen et al., 2011). The combined results from both populations suggest that higher temperatures promote further developed stages of gametogenesis, with the greatest proportion of ripe and spent oysters in both sexes observed earliest in 2011 (CH), followed by 2014 (LR) and then 2013 (LR), which corresponds with the order of average temperatures over those summer periods from the highest (2011) to lowest (2013). Temperature therefore appears to be a key driver of gametogenic progression within *O. edulis* in wild populations, as well as in experimental set ups (Joyce et al., 2013). This is in contrast to the apparent seasonal synchrony in spawning over broadly separated populations observed in the non-native oyster *Crassostrea gigas* in Europe (Philippart et al., 2012). When temperature falls

below a threshold value during late autumn, *O. edulis* gonads appear to enter an over-wintering inactive stage; however, if this threshold is not reached, such as in southern Spain, the gametogenic cycle is not interrupted (Ruiz et al., 1992).

4.2. Sex ratios

Previous research predicted sex ratios of 1:1 (male: female) in *O. edulis* populations (Kamphausen et al., 2011; University Marine Biological Station Millport, 2007), but it is not clear whether that is realistic or stable in healthy natural populations. *Ostrea edulis* is an alternating hermaphrodite and therefore sex changes can occur frequently during the breeding period, resulting in potentially fluctuating sex ratios across the population (Walne, 1974). Interestingly, within this study, only 2.5% of oysters (12 individuals of 480 sampled in 2013/14) were sampled mid-change between the sexes, suggesting that alternation occurs rapidly and there is no intermediate simultaneous hermaphroditic condition or that the alternation occurs infrequently. The high percentage of brooding oysters in LR indicates favourable conditions for *O. edulis* and could support rapid alternation but further research is required in the laboratory to determine this.

There are extensive data already established for the observed sex ratios of *O. edulis* in the waters of Britain and Ireland, though frequently individual point measurements of sex ratios have been taken to represent the long-term male/female bias in a population, for example sex parity within populations has only been recorded during specific periods of the year (Millar, 1964) or in aquarium conditions (Cole, 1939; Mann, 1979). The majority of natural studies indicated a male-skewed ratio of 1.5:1 in cultivated oysters (Bourne, 1890) or between 1.2:1 (Bourne, 1890) and up to 3:1 in natural populations (Cole, 1942; Millar, 1964). More recent investigations found strongly male-dominated populations in a Greek cohort (3:1) (da Silva et al., 2009) and Southampton (6:1) (Kamphausen et al., 2011). The pooled annual sex ratio of males to females in LR was 1.9:1 in 1960, 2.2:1 in 1961 (Millar, 1964), 1.5:1 in 2013 and 2:1 in 2014. Millar (1964) found that male-phase oysters outnumbered female-phase oysters throughout the breeding season in 1960 and

1961. By contrast, although the pooled annual sex ratio in LR was male-biased in both 2013 and 2014, the population maintained a sex ratio which did not depart significantly from parity for most of the time (10 of the 13 months sampled during this period). In CH, where the pooled annual sex ratio was 3:1, sex ratios were consistently more skewed than 2:1 throughout 2011. These results suggest that the LR population is less skewed and significantly different from the other populations, such as those studied in Southampton (Kamphausen et al., 2011), Chichester and a Greek cohort (da Silva et al., 2009) (Table 3).

The sex ratio of a population is vital to ensure breeding success but the impact of a skewed ratio is also dependent on population density and subsequent larval settlement and survival. For example, oysters in a healthy reef are typically densely aggregated, therefore a predominance of males would have a reduced impact on reproductive output because, although there are fewer females, these are highly likely to be fertilised and produce larvae as a consequence of their close proximity to males and an increased substrate for larval settlement and survival. Although a predominance of males could compensate for sperm dilution in populations that have low densities, we found no significant relationship between the proportions of males and brooding females (Figure 7D). So in wild populations under fisheries stress, a male-skewed sex ratio does not appear to be an effective compensatory response.

4.3. Brooding

Ostrea edulis differs from most other oyster species such as *Crassostrea* spp., where ample phenology information is available, by being a larviparous brooder. The proportion of brooding females in a population is a useful measure of reproductive output in *O. edulis*, particularly when supported by data on brood size. The overall percentages of brooding oysters in LR (20% in 2013 and 8% in 2014), were consistently higher than other recently documented UK rates and, according to Walne (1974), within the suggested range for healthy populations. In comparison, only 6.1% of oysters (translocated from LR) sampled in 2014 in Mumbles Bay, Wales were found

to be brooding (Syvret, Woolmer & Eagling, 2015) and in Lough Foyle (Northern Ireland) the proportion of the population brooding has been < 8% over the past three years (Bromley, 2015). However, the proportion of oysters brooding was low in comparison with historical reports from other sites such as West Mersea, Essex (45%) and the Fal Estuary, Cornwall (44%) (Orton, 1927) and showed inter-site and inter-year differences. Although recording the proportion of brooding individuals within a population provides a useful baseline for measuring reproductive success, we suggest this should be combined with sex ratio data in order to assess what percentage of the female population is brooding. To illustrate how this could provide greater insight into population viability, we can consider a theoretical oyster population of 90 individuals where 10% of the population are known to be brooding. This appears to be a relatively low value, however, if we then discover that the sex ratio is 1:8 (10 females and 80 males), this represents successful fertilisation of 90% of females within the population. With the benefits of such insight, appropriate strategies can be developed to aid sustainable management of populations.

4.4. Factors affecting sex ratio

The sex ratio within a population may also skew seasonally in relation to local environmental conditions, with a particular sensitivity to water temperature. This has been noted in many ostreid species, such as *C. gigas* where sex determination is strongly influenced by temperature with a genetic component facilitating male skewed ratios at high temperatures (Santerre et al., 2013). In our study, significantly male skewed sex ratios were observed in LR, following a temperature spike. Previous studies also found that temperature has an effect on sex ratios in both *O. edulis* and *C. gigas* (Joyce et al., 2013; Mann, 1979; Santerre et al., 2013) with more females observed during periods of lower temperatures. Post spawning oysters predominantly observed as male may relate to low metabolic reserves. Mann (1979) found that when *O. edulis* were kept at 18°C or above, the sex ratio was significantly skewed towards males. Millar (1963, 1964) also found the most male skewed ratios in LR occurred when the water temperature was

over 16°C (Figure 5). These previous findings support evidence from this study that *O. edulis* populations may have a critical temperature threshold for sex determination. Higher temperatures were observed in Chichester Harbour, England where the sex ratio was consistently more skewed than 2:1 and similar temperatures were observed in Southampton where the sex ratio was observed to be 6:1 (Kamphausen et al., 2011). The significant increase in the proportion of males with increasing temperature, demonstrated by the present study, supports a strong temperature component in sex determination. This temperature effect may also explain the decline in the proportion of female-phase oysters in LR during the 1960s (Millar, 1964), where temperatures were warmer in the build up to summer. This decline was not evident in LR in the more recent study, although there is an indication of increasing proportions of females towards the end of the breeding period (Figure 5) and also in CH as temperatures declined.

Alongside the relatively well documented effects of temperature on oyster sex ratios (Mann, 1979; Santerre et al., 2013), there are other factors that may influence the sex ratio in *O. edulis*. These include endocrine disrupting chemicals (ECDs), such as tributyltin (TBT) (Kamphausen et al., 2011; Park et al., 2012; Thain & Waldock, 1986) or stress which could be induced by disease (da Silva et al., 2009), shell damage (Bahr & Hillman, 1967; Davis & Hillman, 1971; Ford & Figueras, 1988), and limited food availability (Kennedy, 1983). Since the most common disease known to affect *O. edulis* is *bonamiasis* (Culloty and Mulcahy, 1996), caused by the protozoan parasite *Bonamia ostreae* has not yet reached the LR population (Hugh-Jones, 2011) and pollution and other stressors are thought to be low at LR, temperature is considered to be a main influencer on the sex ratio here. This study site can provide a baseline for the restoration of other healthy UK sites to sustainable levels.

The sustainability of exploited oyster populations is itself dependent on many factors including broodstock density, fertilisation success, and the subsequent larval settlement and survival. The sex ratio of populations play an important role in their long-term sustainability, although these

impacts will vary significantly depending on the population dynamics and factors such as broodstock density, fertilisation success, and the subsequent larval settlement and survival. A strongly biased sex ratio within dispersed populations, such as those in the Solent, England have led to these fishing beds being temporarily closed to fishing due to low oyster density (Southern IFCA pers. comm., 2016). Broodstock density itself is also influenced by fishing pressure, disease (Cochennec, Le Roux, Berthe, & Gerard, 2000) and environmental stress, such as atypically cold winters (Orton, 1940). Furthermore, any larvae produced must survive to settlement, dependent on predation, the availability of food and suitable settlement substrata. Through active management, the sustainability of populations can be improved e.g. through restocking, relaying beds (Eagling, 2015), relaying shell (cultch) to provide suitable substrate for larval settlement and management of fishing pressures (Eagling et al., 2015). The sex ratio in LR was skewed but the population appears to be sufficiently densely laid to maintain a high percentage of brooding oysters. For current restoration projects, we would suggest that striving to achieve the perceived ideal 1:1 sex ratio is unlikely to be successful as this is strongly related to ambient conditions that cannot be controlled.

5. Conclusions

Life-history theory attempts to predict the relative influences of density-dependent and independent influences on life cycles and population dynamics (Winemillar, 2005). Although density-dependent factors are likely to be the major cause of the decline and collapse of many fisheries, including those for oysters (Kamphausen, 2012; Laing, Walker, & Areal, 2005), here we propose that such effects will be exacerbated by density-independent factors, such as temperature. Under predicted climate change scenarios, the shelf sea temperature around the UK is projected to increase by $1.5 < 4^{\circ}\text{C}$ by the end of the 21st century (Jenkins et al., 2010). From the results of this study we suggest that initially, small increases in temperature may create a

longer viable brooding period for *O. edulis*; however, when sea temperatures exceed 16.5°C in LR, the percentage of brooding females declined. This may represent a critical temperature threshold for sex determination, however this may vary between sites and further work will be required to assess how future climate change scenarios may affect the sex ratio of *O. edulis* populations. We recommend that future conservation efforts should be concentrated on creating refuge populations, particularly within MPAs where *O. edulis* are priority marine features for MPA site selection within Scotland. With careful site selection for refuge populations at cooler climes, it may be possible to build long-term, sustainable populations in case of higher water temperatures under extreme climate change scenarios.

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525

Tables

Table 1. Monthly number of male, female and undetermined oysters observed, monthly sex ratio, results of Chi squared tests comparing monthly/total sex ratio to 1:1, for both the summer of 2013 and 2014 in Loch Ryan, Scotland.

Month	Male	Female	Undetermined	Male: Female	Chi squared test compared to 1:1 (χ^2 , df, p)
April	3	3	1	1:1	0, 1, NS
May	N/A	N/A		N/A	N/A
June	23	5	5	4.6:1	11.57, 1, <0.001
July	10	7	4	1.5:1	0.53, 1, NS
August	23	14	3	1.6:1	2.19, 1, NS
September	14	19	7	0.7:1	0.76, 1, NS
October	14	6	15	2.3:1	3.2, 1, NS
Total 2013	87	54	35	1.5:1	7.72, 1, <0.01
April	6	10		0.6:1	0.6, 1, NS
May	24	13	3	1.8:1	3.2, 1, NS
June	10	9	1	1.1:1	0.05, 1, NS
July	23	15	1	1.5:1	1.68, 1, NS
August	14	4	3	3.5:1	14.24, 1, <0.001
September	47	10	2	4.7:1	19.88, 1, <0.001
October	11	7	22	1.6:1	0.4, 1, NS
Total 2014	135	68	32	2:1	26.96, 1, <0.001
Total	222	122	67	1.8:1	29.07, 1, <0.001

Table 2. Monthly percentage of male and female oysters observed, monthly sex ratio, results of Chi squared tests comparing monthly sex ratio to 1:1 for both the summer of 1960 and 1961 in Loch Ryan, Scotland (Adapted from Millar, 1964)

Date	% Male	% Female	Male: Female	Chi squared test compared to 1:1 (χ^2 , df, p)
03/05/1960	64	36	1.77:1	7.84, 1, <0.01
06/06/1960	60	40	1.5:1	4, 1, NS
27/06/1960	71	29	2.45:1	17.64, 1, <0.001
23/07/1960	74	20	3.7:1	31, 1, <0.001
15/08/1960	52	12	4.33:1	25, 1, <0.001
02/10/1960	21	17	1.24:1	0.42, 1, NS
17/12/1960	7	27	0.26:1	11.7, 1, <0.001
25/03/1961	24	35	0.69:1	1.7, 1, NS
03/05/1961	58	40	1.45:1	3.3, 1, NS
31/05/1961	68	31	2.19:1	12.96, 1, <0.001
23/06/1961	66	34	1.94:1	10.24, 1, <0.01
30/06/1961	66	34	1.94:1	10.24, 1, <0.01
10/07/1961	66	19	3.47:1	24.6, 1, <0.001
21/07/1961	56	25	2.24:1	10.97, 1, <0.001
05/08/1961	53	28	1.89:1	7.2, 1, NS
17/08/1961	66	19	3.47:1	24.6, 1, <0.001
22/08/1961	64	9	7.11:1	43.5, 1, <0.001
26/08/1961	43	11	3.91:1	18.9, 1, <0.001

Table 3. Chi squared test results comparing annual variation within Loch Ryan. As results were not significantly different these data were then pooled and the ratio tested against Southampton (Kamphausen et al., 2011), Chichester and Greek populations (da Silva et al., 2009).

Test population	Male: Female	Chi squared compared to Loch Ryan (χ^2 , df, p)	Chi squared compared to 1:1 (χ^2 , df, p)
Loch Ryan 2013 - 2014	3:2 to 2:1	1.9, 1, NS	N/A
Southampton	6:1	39.6, 1, <0.001	71.43, 1, <0.001
Chichester	3:1	5.9, 1, <0.05	47.64, 1, <0.001
Greek cohort	3:1	5.9, 1, <0.05	47.64, 1, <0.001

545

546 Figure Legends

547

548 Figure 1. Map of the United Kingdom and Ireland, black dots indicate the location of the study
549 sites. Adapted from Eagling, Ashton & Eagle, 2015.

550

551 Figure 2. *Ostrea edulis* gonad index showing; Early development male (A) and female (B), Late
552 development male (C) and female (D), Early maturation male (E) and female (F), Ripe male (G)
553 and female (H), Partially spent male (I) and female (J), Spent male (K), Inactive (L) all at 20x
554 magnification.

555

556 Figure 3. Average daily temperature from Loch Ryan, Scotland in 1960, 1961, 2013, 2014 and
557 Chichester Harbour, England 2011

558

559 Figure 4. Gametogenic progression during the breeding period for Loch Ryan, Scotland oysters
560 over 2013 (A) and 2014 (B), and Chichester Harbour, England 2011 (C)

561

562 Figure 5. Seasonal changes in sex ratio expressed as percentages of males (black) and females
563 (grey) overlaid with daily average temperature for Loch Ryan, Scotland in (A) 1960 (B) 1961, (C)
564 2013, (D) 2014 and (E) Chichester Harbour, England in 2011. * represent sex ratios significantly
565 skewed from a 1:1 ratio. Black line indicates predicted threshold temperature 16.5°C in Loch Ryan
566 and 17.5°C in Chichester Harbour

567

568 Figure 6. Seasonal changes in the percentage (of all oysters sampled) of oysters brooding for Loch
569 Ryan, Scotland in (A) 1960, (B) 1961, (C) 2013, (D) 2014 and (E) Chichester Harbour, England in
570 2011. Solid line – Mean annual brooding percentage, Dashed lines – standard error

571

572 Figure 7. Proportion of males (A) and females brooding (B) in relation to average monthly
573 temperature at each sampling interval for Loch Ryan, Scotland 2013/14 and Chichester Harbour,
574 England 2011. (C) Relationship between brood size for *Ostrea edulis* in 2013 & 2014 from Loch
575 Ryan, Scotland and average daily temperature, and (D) Relationship between the proportions of
576 females brooding and males at each sampling interval for Loch Ryan, Scotland 2013/14 and
577 Chichester Harbour, England 2011

578